Adult sex ratios of loggerhead sea turtles (*Caretta caretta*) in two Mediterranean foraging grounds

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**Summary**: Sea turtles show temperature-dependent sex determination (TSD) and information on sex ratios at different life stages is necessary both for population dynamics models for conservation and to shed light on the possible adaptive value of TSD. Adults represent the less abundant class of sea turtle populations and adult sex ratios at foraging grounds are very difficult to obtain. We first analysed biometric data of 460 juvenile and adult loggerhead sea turtles ranging from 60 to 97.5 cm curved carapace length (CCL), in which a clear bimodal distribution of tail length (the main secondary sexual character of adult males) was observed in the size class >75 cm CCL. We then sexed 142 adult turtles in this size class collected from the Tunisian shelf and from the southeastern Tyrrhenian Sea, observing a proportion of females of 51.5% (95% CI: 41.2-61.8%; n=97) and 40.0% (95% CI: 25.7-55.7%; n=45) respectively. Our results complement previous studies and support their findings of similar and more balanced sex ratios in adult and juvenile loggerhead turtles in the Mediterranean, in contrast with highly female-biased sex ratios of hatchlings.

**Keywords**: *Caretta caretta*; sea turtle; adult; sex ratio; maturity; temperature-dependent sex determination; Mediterranean.

**INTRODUCTION**

The sex of sea turtles is determined by the temperature to which an embryo is exposed during its development (Wibbels 2003), as in many other reptiles (Janzen and Paukstis 1991). In general, skewed sex ratios are more common in species with temperature-dependent sex determination (TSD) than in species with a geno-
typic sex determination (Bull 1980). Sea turtles are no exception and female-biased sex ratios are observed in most cases (Wibbels 2003). The adaptive value of TSD and skewed sex ratios is puzzling. Fisher (1930) elegantly explained why natural selection favours equal and skewed sex ratios is puzzling. Fisher (1930) elegantly explained why natural selection favours equal sex ratios among species and populations is necessary to shed light on the evolutionary basis of TSD in sea turtles (Mrosovsky and Provanca 1992). A good sampling of natural sex ratios and possible sex ratio variability among species and populations is necessary to shed light on the evolutionary basis of TSD in sea turtles (Mrosovsky 1994, Freedberg and Wade 2001). Sex ratio must also be considered in population dynamics of species with TSD, since important parameters such as population size and reproductive output can only be estimated if several demographic parameters, including sex ratio, are available for the models (e.g. Heppell et al. 2003). Since sea turtles are species of conservation concern, this is particularly important for understanding how they may respond to both anthropogenic threats and conservation measures. Unfortunately, sex ratio is not easy to assess in sea turtles, and this leads to increased uncertainty in population models.

For convenience, three major life stages (hatchlings, juveniles, and adults) can be distinguished when one is investigating sea turtle sex ratios and different methods are used for different stages. Hatchling sex ratio is the most easy to obtain, either directly by examining their gonads (e.g. Yntema and Mrosovsky 1980), or indirectly from nest temperature or other variables associated with nest temperature (e.g. Mrosovsky et al. 1999). Juveniles can be sampled in relatively high numbers when they strand, are incidentally caught in fishing gear or are directly captured at sea. Then they can be sexed using different methods such as blood hormonal dosage, histology and direct observation of gonadal morphology by laparoscopy or during necropsies (Wibbels 1999). Only adults show external sexual dimorphism, notably an elongated tail in males, so it is relatively easy to sex them (Casale et al. 2005). Unfortunately, they represent the least abundant class of sea turtle populations, so sampling adults at foraging areas is intrinsically difficult. Adults can be found in high numbers and densities when they aggregate at mating sites near nesting beaches during the breeding season (e.g. Schofield et al. 2009). However, the sex ratios observed at these sites (operational sex ratios) may not represent the actual adult sex ratio of their population because of possible different breeding periodicity of males and females (Miller 1997, Stewart and Dutton 2011, Wright et al. 2012). Therefore, more reliable adult sea turtle sex ratios can be assessed at foraging grounds where, however, they are very difficult to collect because of their low abundance. For these reasons, juvenile sex ratios, adult sex ratios at foraging grounds and operational sex ratios should be investigated separately.

The loggerhead turtle (Caretta caretta) is the most abundant sea turtle species in the Mediterranean, and reproduces mainly in Greece, Turkey, Cyprus and Libya (Casale and Margaritoulis 2010). Loggerhead turtles frequent the entire marine area of the Mediterranean Sea. Oceanic zones are mainly frequented by small juveniles and a high occurrence is reported in the westernmost part of the basin (from the Alboran Sea to the Balearic Islands), the Strait of Sicily, and the Ionian Sea. Larger juveniles and adults tend to frequent neritic zones, with high occurrence observed in the north Adriatic, off Tunisia-Libya, off Egypt, and off the southeast coast of Turkey (Casale and Margaritoulis 2010). One of the most distinctive characteristics of the Mediterranean population is the significantly smaller adult size in comparison with other populations around the world (Dodd 1988, Tiwari and Bjorndal 2000, Margaritoulis et al. 2003): on average Mediterranean loggerhead turtles mature at a size larger than 70 cm curved carapace length (Margaritoulis et al. 2003, Casale et al. 2005).

The first attempt to estimate sex ratios of adult loggerhead sea turtles in the Mediterranean was based on individuals collected from a wide area all around the Italian peninsula (Casale et al. 2005). More recently, genetic markers showed that individuals originating from different nesting sites distribute differently among neritic foraging areas, i.e. turtles from a certain rookery prevalently frequent certain areas and turtles from another rookery prevalently frequent other areas (Garofalo et al. 2013, Clusa et al. in press). Moreover, satellite tracking showed a high degree of fidelity of loggerhead turtles, in particular adults, to specific neritic areas (Broderick et al. 2007, Schofield et al. 2010, Casale et al. 2012, Casale et al. 2013, Rees et al. 2013). Therefore, different adult sex ratios can be associated with different neritic areas and should be preferably assessed at local level first, then at regional level. As said, the most limiting factor is the rarity of adults at foraging grounds and so far one study has reported an adult sex ratio from a specific foraging ground: the Gulf of Amvrakikos, Greece (Rees et al. 2013). Operational sex ratios have been investigated only at one breeding site (Zakynthos, Greece), where a relatively balanced operational sex ratio was estimated (Hays et al. 2010).

Genetic markers (Garofalo et al. 2013), tag returns (Margaritoulis et al. 2003) and satellite tracking (Broderick et al. 2007, Casale et al. 2013, Schofield et al. 2013) showed that the continental shelf off Tunisia and Libya is one of the most important neritic foraging grounds in the Mediterranean and is frequented by loggerhead juveniles and adults originating from different Mediterranean breeding sites in Greece, Libya, and Cyprus. In the western Mediterranean, loggerhead turtles are also commonly encountered along the southwestern coasts of Italy in the Tyrrenhian Sea. Mediterranean juveniles and adults utilize the rich local swallow habitats of this area to forage (Hochscheid et al. 2013, Maffucci et al. 2013, Clusa et al. in press). This study aims to provide estimations of adult sex ratios of loggerhead turtles at the above two foraging grounds.
A total of 505 loggerhead turtles were considered in this study. They were incidentally captured by fishing gear (n=419), found stranded (n=32), found floating at sea (n=52), or found while nesting (n=2). They were collected from two areas: the waters around Lampedusa island, Italy, on the Tunisian shelf, in the period 1991-2012 (n=460), and the southeastern Tyrrhenian Sea in the period 2000-2013 (n=45) (Fig. 1). The above sampling methods, except nesting, are assumed not to be sex-biased, i.e. the probability of being incidentally captured, or of stranding or to be found floating at sea in foraging areas is the same for both sexes. However, for 16 turtles this assumption was not valid; they were therefore excluded from sex ratio analysis (see below) and were only considered for setting up the sexing method. Sample size prevented interannual sex ratio differences from being assessed and for convenience sex ratios were assumed to be constant during the study period.

These data were used in a three-step process aimed at estimating adult sex ratios. The first step analysed biometric data of turtles in the size range of juveniles and adults in order to assess a good threshold for the adult size, i.e. the size above which almost all turtles are mature and a turtle with a short tail (the main sexually dimorphic character) is unlikely to be an immature male and is likely to be a mature female. In order to improve detection of the appearance of the main sexually dimorphic character (tail length) and to interpret this character also in the light of reference values for adult females recently made available (Rees et al. 2013), we analysed biometric data of 460 loggerhead turtles ranging from 60 to 97.5 cm curved carapace length (CCL). This part of the study was conducted only in one area, the Tunisian shelf, where a suitable sample of turtles was available. We limited the analysis to the range >60 cm CCL because previous studies (Casale et al. 2005, Rees et al. 2013) showed that on average loggerhead males begin to develop an elongated tail around 65 cm of CCL notch-to-tip (Bolten 1999), so the threshold size for adulthood is expected to be above 65 cm CCL. The second step determined a good threshold of tail length for sexing adults, i.e. the tail length above and below which a turtle can be considered as male or female, respectively. This threshold value was determined on the basis of the bimodal distribution of tail lengths observed in the adult size class and compared with previous similar studies in the region (Casale et al. 2005, Rees et al. 2013). The third step estimated sex ratios of 142 turtles in the adult size class (97 from the Tunisian shelf and 45 from the Tyrrhenian Sea), after removing 16 turtles (9 males and 7 females) because their finding was probably not independent from their sex. Turtles were sexed either by tail length, as described above, or by other methods, as follows. All turtles collected from the Tunisian shelf (all alive; n=97) were sexed according to the distance from the posterior margin of the carapace to the tip of the tail (carapace-tail) and the distance from the posterior margin of the carapace to the cloaca (carapace-cloaca), which were previously proposed as the best indicators of sex in adult individuals (Casale et al. 2005). Twelve live turtles collected in the Tyrrhenian were sexed according to one of the above tail measures, depending on the individual cases. In four of these turtles, photographs were used to determine whether carapace-cloaca was >0 (the sexing threshold), i.e. whether the cloaca was internal or external to the carapace, which can be easily determined by eye. The sex of 33 other dead turtles from the Tyrrhenian was determined through visual examination of the gonads during necropsies. Before release, all live turtles were tagged with metal or plastic flipper tags (Balazs 1999) to avoid replication.

Adult sex ratios at foraging grounds can be biased by sex-specific breeding periodicity, and to avoid this bias it is preferable to estimate sex ratios outside the breeding period (Wibbels 2003). However, in the Mediterranean adult males start migrating to breeding sites as early as October and migrate back to foraging grounds towards May (Hays et al. 2010, Schofield et al. 2010, Casale et al. 2013), while adult females arrive at breeding sites towards April (Hays et al. 2010) and return to foraging grounds as late as October (Zhinden et al. 2011). Hence, one sex or the other is always expected to be underrepresented at foraging grounds. In order to consider potential seasonal differences in sex ratios, we also calculated sex ratios for two periods: when more males are expected at foraging grounds (Jun-Sep) and when more females are expected (Oct-Mar).

We calculated 95% confidence intervals of sex ratios according to the method for binomial distributions (Zar 1999). Pairwise statistical tests between seasons and among known sex ratios of hatchling, juvenile and adult loggerheads in the Mediterranean were conducted by Fisher exact test on 2×2 contingency tables with the observed numbers of individuals. CCL values were...
Fig. 2. – Distribution according to curved carapace length of two measures of the tail (carapace-tail and carapace-cloaca) of 460 loggerhead sea turtles from the Tunisian shelf, central Mediterranean.

RESULTS

In the sample from the Tunisian shelf, the distribution of carapace-tail and carapace-cloaca values by CCL (Fig. 2) and the frequency distribution of these two measures in different 5-cm CCL classes (Fig. 3) show that an elongated tail starts to be common at 65-75 cm CCL, but a clear bimodal distribution only arises at >75 cm CCL. Comparison between the 75-80 cm CCL and >80 cm CCL size classes (Fig. 3) indicates that the two sexes are similarly differentiated in both size classes, so adopting 75 cm as the adult threshold is the best trade-off between sample size and sexual dimorphism.

On the basis of the observed bimodal distributions, good threshold values for determining sex are 5 cm and 0 cm for carapace-tail and carapace-cloaca, respectively, as previously suggested (Casale et al. 2005). This is consistent with the maximum tail length (7 cm) observed among 94 nesting females (Rees et al. 2013), since the latter measure was taken from the notch between the supracaudal scutes (vs. the tip of the supracaudal scutes in this study) and therefore it is slightly longer (ca. 1-3 cm) than the measure considered in this study (carapace-tail).

The proportion of females among turtles >75 cm CCL was 51.5% (95% CI: 41.2-61.8%; n=97) in the Tunisian shelf sample and 40.0% (95% CI: 25.7-55.7%; n=45) in the Tyrrhenian sample, and the two were not found to be significantly different (Fisher exact test; p=0.17; n=142). No significant differences were observed between the two periods of the year (Jun-Sep and Oct-Mar) within the same sample: 46.0% (95% CI: 33.4-59.1%; n=63) in Jun-Sep and 61.8% in Oct-Mar (95% CI: 43.6-77.8%; n=34) in the Tunisian shelf sample (Fisher exact test; p=0.20; n=97) and 50.0% (95% CI: 28.2-71.8%; n=22) in Jun-Sep and 31.6% (95% CI: 12.6-56.6%; n=19) in Oct-Mar in the Tyrrhenian sample (Fisher exact test; p=0.34; n=41). However, while the sex ratios from the two areas were similar in Jun-Sep (Fisher exact test; p=0.81; n=85) they were different in Oct-Mar, with fewer females in the Tyrrhenian sample (Fisher exact test; p<0.05; n=53).

DISCUSSION

The two sex ratios observed in this study are not significantly different from the other adult sex ratios in single foraging grounds (Ionian Greece) or from juvenile sex ratios in different areas, except for the adult vs. juvenile sex ratios in the Tyrrhenian Sea (Table 1). However, they differ from one or two (depending on the study area) hatching sex ratios for which a statistical comparison is possible (Table 1). On the basis of incubation duration, Godley et al. (2001a) hypothesized that a female-biased hatching sex ratio is produced in most of the major nesting sites of the Mediterranean. In fact, all the specific studies conducted so far in major nesting sites in Greece, Turkey and Cyprus estimated a high female proportion (Table 1). On the other hand, juvenile sex ratios appear to be more balanced, and adult sex ratios are even male-biased in two cases: in the Tyrrhenian and in the Amvrakikos Gulf (Table 1). However, these two cases are probably different, because in the Tyrrhenian the male bias was only observed in the period October-March, while the sex ratio in the Amvrakikos Gulf refers to the period May-September, this being the only period sampled there (Rees et al. 2013). Therefore, data from the Amvrakikos Gulf and from the Tunisian shelf in the period June-September are somehow in line with the expectations: less females in June-September at foraging grounds (see above). By contrast, the male bias observed in the Tyrrhenian in the period Oct-March is intriguing and deserves further investigation. Sample size may limit the capability of detecting differences in some cases, and larger samples from different seasons and foraging areas are needed to unveil adult sex ratio patterns and their possible differences from juvenile sex ratios in the Mediterranean. On the other hand, there is an obvious difference between the hatching and juvenile/adult sex ratios known so far. A similar discrepancy has also been observed in the northwest Atlantic (Wibbels 2003). Three general cases can explain this discrepancy: (i) the available sex ratios are representative of the population demography and post-hatching/juvenile females experience a higher mortality than males; (ii) the current juvenile and adult sex ratios are not representative of the entire population and higher female-biased sex ratios occur in foraging areas not yet investigated; (iii) the current hatching sex ratios are not representative of the entire population and more balanced or male-biased sex ratios are produced in under-studied periods or beach
sectors, or at other nesting sites, including minor sites and areas with diffuse nesting.

Adult sex ratios at breeding grounds (operational sex ratios) will reflect sex ratios at foraging grounds only if the breeding periodicity of males and females is similar. In the Mediterranean, operational sex ratios have been investigated only at the Zakynthos breeding site (Greece) where a relatively balanced operational sex ratio (43% females) is estimated (Hays et al. 2010). This value is fairly similar to the sex ratios observed at the foraging grounds investigated so far and would suggest a similar breeding periodicity of males and fe-
males. However, there are indications that males breed more often than females (Hays et al. 2010), although males. However, there are indications that males breed more often than females (Hays et al. 2010), although males. However, there are indications that males breed more often than females (Hays et al. 2010), although males. However, there are indications that males breed more often than females (Hays et al. 2010), although males. However, there are indications that males breed more often than females (Hays et al. 2010), although males. However, there are indications that males breed more often than females (Hays et al. 2010), although males. However, there are indications that males breed more often than females (Hays et al. 2010), although males. However, there are indications that males breed more often than females (Hays et al. 2010), although males. However, there are indications that males breed more often than females (Hays et al. 2010), although males. However, there are indications that males breed more often than females (Hays et al. 2010), although males. However, there are indications that males breed more often than females (Hays et al. 2010), although males. However, there are indications that males breed more often than females (Hays et al. 2010), although males. However, there are indications that males breed more often than females (Hays et al. 2010), although males. However, there are indications that males breed more often than females (Hays et al. 2010), although males. However, there are indications that males breed more often than females (Hays et al. 2010), although males. However, there are indications that males breed more often than females (Hays et al. 2010), although males. However, there are indications that males breed more often than females (Hays et al. 2010), although males. However, there are indications that males breed more often than females (Hays et al. 2010), although males. However, there are indications that males breed more often than females (Hays et al. 2010), although males. However, there are indications that males breed more often than females (Hays et al. 2010), although males. However, there are indications that males breed more often than females (Hays et al. 2010), although males. However, there are indications that males breed more often than females (Hays et al. 2010), although males. However, there are indications that males breed more often than females (Hays et al. 2010), although males. However, there are indications that males breed more often than females (Hays et al. 2010), although males. However, there are indications that males breed more often than females (Hays et al. 2010), although males. However, there are indications that males breed more often than females (Hays et al. 2010), although males. However, there are indications that males breed more often than females (Hays et al. 2010), although males. However, there are indications that males breed more often than females (Hays et al. 2010), although males. However, there are indications that males breed more often than females (Hays et al. 2010), although males. However, there are indications that males breed more often than females (Hays et al. 2010), although males. However, there are indications that males breed more often than females (Hays et al. 2010), although males. However, there are indications that males breed more often than females (Hays et al. 2010), although males. However, there are indications that males breed more often than females (Hays et al. 2010), although males. However, there are indications that males breed more often than females (Hays et al. 2010), although males. However, there are indications that males breed more often than females (Hays et al. 2010), although

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